Purification, Crystallization and Preliminary X-Ray Diffraction Studies of Xanthine Dehydrogenase and Xanthine Oxidase Isolated from Bovine Milk

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Introduction: Xanthine oxidase (XO) is an archetypal enzyme, which was originally described as aldehyde oxidase by Schardinger, 1902, and subsequently identified as xanthine oxidase by Morgan in 1922. It is now clear that the enzyme can catalyze the oxidation of a wide range of substrates including purines, pyrimidines and aldehydes. Xanthine oxidoreductases have been isolated from a wide range of organisms, from bacteria to man with sequence identities ranging from 98 % to the low 40 %. So far, no full-length homologues have been found in archaeal organisms. All of the enzymes have similar molecular weights and bind the same series of redox centers, Mo-pterin, two iron-sulfur centers, and FAD. The bovine milk xanthine oxidoreductase is still the best characterized of the molybdenum-containing flavoproteins, and work on the enzyme is at the forefront of physico-chemical and kinetic studies of this important class of biocatalysts.

Methods and Materials: The final conditions for growing the best XO crystals, not containing the cryoprotectant, were as follows: The XO stock solution was diluted with 40 mM Tris pH 7.5, 20 mM pyrophosphate pH 8.5, 5 mM DTT, 1 mM salicylate and 0.2 mM EDTA (Buffer B) to a concentration of 24 mg/ml. The precipitant solution contained 50 mM KP_i pH 6.5, 5 mM DTT, 1 mM sodium salicylate, and 0.2 mM EDTA (Buffer C) and a final concentration of 18 % (w/v) PEG 4000. In addition, crystals of both XO and XDH could be produced in crystallization solutions, which contained a glycerol concentration sufficient to allow flash freezing of the crystals. For the enzyme solution, the stored enzyme stock was diluted with Buffer B to a final concentration of 20% glycerol and 18mg/ml enzyme for XO or 30% glycerol and 10mg/ml enzyme for XDH. The optimized conditions for the precipitant solution were Buffer C containing either 23% PEG 4000 and 20% glycerol for the XO crystals, or 22% PEG 4000 and 30% glycerol for the XDH crystals. Protein and precipitant solutions were filtered and equal volumes (20 ul each) of both were mixed to start crystallization.

Initially, data sets for XO were collected from three different crystals at 22 °C and a wavelength of 1.000 Å at beamline X8C, NSLS, Brookhaven National Laboratories, on a mosaic 4 K CCD detector (ADSC, San Diego, CA). XDH diffraction data were collected from a single crystal that was broken away from one of the clusters produced with this enzyme form. After flash-freezing, the crystal was held at a temperature of 100 K on beamline X8C, NSLS, Brookhaven National Laboratory, again using a 4 K CCD and the X-ray wavelength set to 1.000 Å for data collection. All data sets were processed and scaled together using the program suite HKL

Results: XO crystallizations with glycerol resulted in separate crystals of up to 200 x 100 x 75 μm. In contrast to the XO crystals, which are of reasonable thickness, the XDH crystals very thin plates, with a typical size of 300 x 300 x 10 um. They are usually grown together in a fan-like shape. The unfrozen XO crystals belong to space group $C222_1$, having unit cell dimensions a = 118.6 Å, b = 165.3 Å, c = 156.4 Å. Bovine XDH crystals belong to space group C2, but show pseudo-orthorhombic symmetry. Their unit cell dimensions are a = 169.9 Å, b = 124.8 Å, c = 148.6 Å and beta = 90.0°. The resolution extends to 2.1 Å with an overall R_{Sym} of 8.1%.

Conclusions: The monoclinic space group of the XDH crystals may be generated by slight changes in the unit cell axes and a shift in one unit cell angle from 90° to 90.9° of the XO crystals. The Matthews parameters are 2.6 and 2.7 for XO and XDH, respectively, indicating one subunit per asymmetric unit in the XO crystals and a complete dimer per asymmetric unit in the XDH crystals

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